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(57) Abstract

Methods of treating conditions characterized by cellular hyperproliferation using Interleukin-1 inhibitory compounds and compounds useful in the described methods are provided.

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DESCRIPTION

Treatment of Cellular Hyper-Proliferation by Inhibition of Interleukin-1

Cross-Reference to Related Applications

The present application is a continuation in part of United States Serial No. 07/707,897 filed May 31, 1991, the disclosure of which is incorporated herein by reference.

Background of the Invention

The present invention is directed to methods of treatment of cellular hyperproliferation. These methods are useful in the treatment of various pathologic conditions characterized by over-proliferation of cells, especially epithelial cells and, more particularly, keratinocytes.

Conditions characterized by hyperproliferation of cells, particularly keratinocytes, have proved difficult to treat. Conventional therapies for some of these conditions such as psoriasis have been unsatisfactory. Thus, there exists a need for therapies which can treat these conditions by decreasing or preventing over-proliferation of cells.

Cytokine levels in certain conditions characterized by cellular hyperproliferation have been studied. In particular, there have been attempts to compare levels of Interleukin-1α and Interleukin-1β in normal and in psoriatic skin. Interleukin-1 activity in psoriatic skin has been reported to be reduced in relation to normal skin. However, in psoriatic skin higher levels of a high molecular weight Interleukin-1β in comparison to Interleukin-1β levels in normal skin form have been reported. (See, Cooper et al., J. Invest. Dermatol. 95(5):245 to 265 (1990)).

Summary of the Invention

The present invention provides methods of preventing or decreasing hyperproliferation of cells, particularly epithelial cells, by inhibiting or decreasing inhibition of Interleukin-1 ("IL-1") activity or intracellular Interleukin-1 receptor antagonist activity. Thus, the present invention is directed to such methods which comprise contacting the cells with a hyperproliferation inhibiting amount of an Interleukin-1 inhibitory compound. Interleukin-1 inhibitory compounds include compounds which decrease Interleukin-1 or intracellular Interleukin-1 receptor antagonist activity. According to one aspect, Interleukin-1 activity is inhibited by inhibiting or down-regulating its synthesis. According to another aspect, Interleukin-1 activity is inhibited by inhibiting conversion of a precursor form into an active form. This inhibition of conversion into an active form may be accomplished by one of several means, including, but not limited to, preventing or decreasing expression of a converting enzyme which converts a precursor form into an active form or by inhibiting the converting enzyme per se. Suitable Interleukin-1 inhibitory compounds include Oligomers (such as antisense Oligomers, Third Strand Oligomers or Triplex Oligomer Pairs) which regulate expression of an Interleukin-1, an Interleukin-1 modulating factor, or an Interleukin-1 converting enzyme. Interleukin-1 modulating factors include compounds which modulate Interleukin-1 activity and include, for example, compounds such as intracellular Interleukin-1 receptor antagonist. Other Interleukin-1 inhibitory compounds may be selected from a variety of classes of compounds which decrease activity levels of Interleukin-1 or intracellular Interleukin-1 receptor antagonist in the targeted (i.e., hyperproliferating) cells.

According to a preferred aspect, the present invention is directed to methods of treating pathologic conditions characterized by hyperproliferation of skin or

epithelial cells by contacting skin or epithelial cells with a proliferation-inhibiting amount of Oligomer(s). The Oligomer may be an antisense Oligomer, a Third Strand Oligomer or a Triplex Oligomer. Such an antisense Oligomer has a sequence complementary to a sequence of RNA transcribed from a target gene present in the cells. A Triple Strand Oligomer has a sequence complementary to a selected double stranded nucleic acid sequence of a target gene present in the cells. A Triplex Oligomer Pair is complementary to a single stranded nucleic acid sequence of a target gene or its transcription product. The target gene is selected from those genes which encode a cytokine which mediates cellular proliferation or a modulating factor (such as a receptor antagonist) thereof or a converting enzyme therefor.

According to an alternate preferred aspect, the present invention is directed to methods of decreasing or preventing hyperproliferation of skin or epithelial cells by contacting the cells with a hyperproliferation-inhibiting amount of an Oligomer which comprises an antisense Oligomer, a Triple Strand Oligomer or a Triplex Oligomer Pair. The Oligomer has a sequence complementary to a nucleic acid sequence from a target gene present in the cells or transcription product. The target gene is selected from those genes encoding cytokines which mediate cellular proliferation, or a modulating factor such as a receptor antagonist for such a cytokine, or a converting enzyme, or an enzyme involved in translational or post-translational modification of the cytokine that is critical for its function.

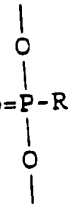
Preferred target genes include those which encode Interleukin-1β ("IL-1β"), Interleukin-1α ("IL-1α"), intracellular Interleukin-1 receptor antagonist ("icIL-1ra" or "intracellular IL-1ra"), or an Interleukin-1 converting enzyme.

Definitions

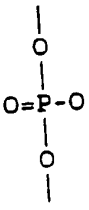
As used herein, the following terms have the following meanings unless expressly stated to the contrary.

The term "purine" or "purine base" includes not only the naturally occurring adenine and guanine bases, but also modifications of those bases such as bases substituted at the 8- position, or to the guanine analogs modified at the 6-position or the analog of adenine, 2-amino purine.

The term "nucleoside" includes a nucleosidyl unit and is used interchangeably therewith, and refers to a subunit of a nucleic acid which comprises a 5 carbon sugar and a nitrogen- containing base. The term includes not only units having A, G, C, T and U as their bases, but also analogs and modified forms of the bases (such as 8-substituted purines). In RNA, the 5 carbon sugar is ribose; in DNA, it is a 2'-deoxyribose. The term also includes analogs of such subunits, including modified sugars such as 2'-O-alkyl ribose.

The term "phosphonate" refers to the group 

wherein R is an alkyl or aryl group. Suitable alkyl or aryl groups include those which do not sterically hinder the phosphonate linkage or interact with each other. The phosphonate group may exist in either an "R" or an "S" configuration. Phosphonate groups may be used as internucleosidyl phosphorus group linkages (or links) to connect nucleosidyl units.

The term "phosphodiester" refers to the group 

wherein phosphodiester groups may be used as internucleosidyl phosphorus group linkages (or links) to connect nucleosidyl units.

A "non-nucleoside monomeric unit" refers to a monomeric unit wherein the base, the sugar and/or the phosphorus backbone has been replaced by other chemical moieties.

A "nucleoside/non-nucleoside polymer" refers to a polymer comprised of nucleoside and non-nucleoside monomeric units.

The term "oligonucleoside" or "Oligomer" refers to a chain of nucleosides which are linked by internucleoside linkages which is generally from about 6 to about 100 nucleosides in length, but which may be greater than about 100 nucleosides in length. They are usually synthesized form nucleoside monomers, but may also be obtained by enzymatic means. Thus, the term "Oligomer" refers to a chain of oligonucleosides which have internucleosidyl linkages linking the nucleoside monomers and, thus, includes oligonucleotides, nonionic oligonucleoside alkyl- and aryl-phosphonate analogs, alkyl- and aryl-phosphonothioates, phosphorothioate or phosphorodithioate analogs of oligonucleotides, phosphoramidate analogs of oligonucleotides, neutral phosphate ester oligonucleoside analogs, such as phosphotriesters and other oligonucleoside analogs and modified oligonucleosides, and also includes nucleoside/non-nucleoside polymers. The term also includes nucleoside/nucleotide polymers wherein one or more of the phosphorus group linkages between monomeric units has been replaced by a non-phosphorous linkage such as a formacetal linkage, a sulfamate linkage, or a carbamate linkage. It also includes nucleoside/non-nucleoside polymers wherein both the sugar and the phosphorous moiety have been replaced or modified such as morpholino base analogs, or polyamide base analogs. It also includes nucleoside/non-nucleoside polymers wherein the base, the sugar, and the phosphate backbone of the non-nucleoside

are either replaced by a non-nucleoside moiety or wherein a non-nucleoside moiety is inserted into the nucleoside/non-nucleoside polymer. Optionally, said non-nucleoside moiety may serve to link other small molecules which may interact with target sequences or alter uptake into target cells.

The term "alkyl- or aryl-phosphonate Oligomer" refers to Oligomers having at least one alkyl- or aryl-phosphonate internucleosidyl linkage. Suitable alkyl- or aryl-phosphonate groups include alkyl- or aryl- groups which do not sterically hinder the phosphonate linkage or interact with each other. Preferred alkyl groups include lower alkyl groups having from about 1 to about 6 carbon atoms. Suitable aryl groups have at least one ring having a conjugated pi electron system and include carbocyclic aryl and heterocyclic aryl groups, which may be optionally substituted and preferably having up to about 10 carbon atoms.

The term "methylphosphonate Oligomer" (or "MP-Oligomer") refers to Oligomers having at least one methylphosphonate internucleosidyl linkage.

The term "neutral Oligomer" refers to Oligomers which have nonionic internucleosidyl linkages between nucleoside monomers (i.e., linkages having no net positive or negative ionic charge) and include, for example, Oligomers having internucleosidyl linkages such as alkyl- or aryl-phosphonate linkages, alkyl- or aryl-phosphonothioates, neutral phosphate ester linkages such as phosphotriester linkages, especially neutral ethyltriester linkages; and non-phosphorus-containing internucleosidyl linkages, such as sulfamate, morpholino, formacetal, and carbamate linkages. Optionally, a neutral Oligomer may comprise a conjugate between an oligonucleoside or nucleoside/non-nucleoside polymer and a second molecule which comprises a conjugation partner. Such conjugation partners may comprise intercalators, alkylating agents, binding substances for cell surface receptors, lipophilic agents,

nucleic acid modifying groups including photo-cross-linking agents such as psoralen and groups capable of cleaving a targeted portion of a nucleic acid, and the like. Such conjugation partners may further enhance the uptake of the Oligomer, modify the interaction of the Oligomer with the target sequence, or alter the pharmacokinetic distribution of the Oligomer. The essential requirement is that the oligonucleoside or nucleoside/non-nucleoside polymer that the Oligomer conjugate comprises be neutral.

The term "neutral alkyl- or aryl- phosphonate Oligomer" refers to neutral Oligomers having neutral internucleosidyl linkages which comprise at least one alkyl- or aryl- phosphonate linkage.

The term "neutral methylphosphonate Oligomer" refers to neutral Oligomers having internucleosidyl linkages which comprise at least one methylphosphonate linkage.

The term "tandem oligonucleotide" or "tandem Oligomer" refers to an oligonucleotide or Oligomer which is complementary to a sequence located either on the 5'- or 3'- side of a target nucleic acid sequence and which is co-hybridized with a second Oligomer which is complementary to the target sequence. Tandem Oligomers may improve hybridization of these Oligomers to the target by helping to make the target sequence more accessible to such Oligomers, such as by decreasing the secondary structure of the target nucleic acid sequence. In addition, one member of a pair of tandem Oligomers may improve the hybrid stability of the second tandem Oligomer to the target nucleic acid sequence by promoting a helical structure at either the 5'- or 3'-end of said second Oligomer and vice-versa.

The term "short chain aliphatic alcohol" refers to an alcohol having from about 2 to about 20 carbon atoms in which the aliphatic (alkyl) chain may be either straight chained or branch chained and includes primary, secondary and tertiary alcohols, glycols and polyols.

The term "flux enhancer" refers to a substance which is used to increase transdermal flux of a compound. A flux enhancer is typically applied to skin or mucous membrane in combination with the compound to increase transdermal flux of the compound. Enhancers are believed to function by disrupting the skin or mucous membrane barrier or by changing the partitioning behavior of the drug in the skin or mucous membrane.

The term "Triplex Oligomer Pair" refers to first and second Oligomers which are complementary to and which are capable of hydrogen bonding to a segment of a single stranded target nucleic acid, such as RNA or DNA, and, thus, together with the single stranded target nucleic acid are capable of forming a triple helix structure therewith.

The term "Third Strand Oligomer" refers to Oligomers which are capable of hybridizing to a segment of a double stranded nucleic acid, such as a DNA duplex, an RNA duplex or a DNA-RNA duplex, and forming a triple helix structure therewith.

The term "complementary," when referring to a Triplex Oligomer Pair (or first and second Oligomers) or to a Third Strand Oligomer, refers to Oligomers having base sequences which hydrogen bond (and base pair or hybridize) with the base sequence of the nucleic acid to form a triple helix structure.

Detailed Description of the Invention

According to the present invention, conditions characterized by hyperproliferation of cells, especially epithelial cells, are treated using a hyperproliferation inhibiting amount of an Interleukin-1 inhibitory compound. Interleukin-1 inhibitory compounds include compounds which decrease Interleukin-1 or intracellular Interleukin-1 receptor antagonist activity. Suitable Interleukin-1 inhibitory compounds include compounds which inhibit or decrease expression of a Interleukin-1, an Interleukin-1

modulating factor, such as intracellular Interleukin-1 receptor antagonist, or an enzyme which converts an IL-1 precursor to an active form ("converting enzyme") and, thus, includes peptides, competitive or non-competitive inhibitors for converting enzymes, small molecule inhibitors, antibodies, Oligomers which bind to a protein's active site to modulate its function, or Oligomers such as antisense Oligomers, Third Strand Oligomers and Triplex Oligomer Pairs. Suitable nucleoside sequences for these Oligomers may be determined from the sequences of target genes. Preferred sequences of the target region are described herein below. Other suitable Interleukin-1 inhibitory compounds include compounds which inhibit a converting enzyme and, thus, prevent conversion of an IL-1 precursor into an active form. Other Interleukin-1 inhibitory compounds include compounds which decrease intracellular Interleukin-1 receptor antagonist activity.

A. Preferred Oligomers

The Oligomer selected may be any of a number of types, including those having a charged or uncharged backbone.

Preferred Oligomers include alkyl- and aryl-phosphonate Oligomers, especially preferred are methylphosphonate Oligomers. Other preferred Oligomers include phosphorothioate Oligomers, morpholino analogs, formacetal analogs and peptide nucleic acid ("PNA") analogs. Also preferred are Oligomers having at least about 8 nucleosidyl units, more preferably from about 8 to about 40 nucleosidyl units. Also preferred are Oligomers which are nucleoside/non-nucleoside polymers. Suitable Oligomers also include chimeric oligonucleotides which are composite RNA, DNA analogues (Inoue et al., FEBS Lett. 2115:327 (1987)). Oligomers having a neutral backbone, for example, methylphosphonate Oligomers with cleaving or cross-linking moieties attached, may prove advantageous in certain circumstances; such Oligomers may have a longer

half-life in vivo since the neutral structure reduces the rate of nuclease digestion while the cleaving or cross-linking moiety may promote inactivation of target polynucleotide sequences.

According to one aspect of the present invention, these antisense Oligomers have a sequence which is complementary to a portion of the RNA transcribed from the selected target gene. Although the exact molecular mechanism of inhibition has not been conclusively determined, it has been suggested to result from formation of duplexes between the antisense Oligomer and the RNA transcribed from the target gene. The duplexes so formed may inhibit translation, processing or transport of an mRNA sequence or may lead to digestion by the enzyme RNaseH.

According to an alternate aspect of the present invention, down regulation of cellular proliferation may be accomplished by triple helix formation using a Third Strand Oligomer or a Triplex Oligomer Pair having sequences selected such that the Oligomer(s) are complementary to and form a triple helix complex with a target sequence of double stranded or single stranded nucleic acid and thereby interfere with or prevent expression of the targeted nucleic acid sequence. Further descriptions of the use of Oligomers (including Third Strand Oligomers and Triplex Oligomer Pairs) to prevent or interfere with the expression of a target sequence of double or single stranded nucleic acid by formation of triple helix complexes is described in the copending U.S Patent Application Serial Nos. 07/388,027, 07/751,813 and 07/772,081, the disclosures of which are incorporated herein by reference.

As a general matter, the Oligomer employed will have a sequence that is complementary to the sequence of the target nucleic acid. However, absolute complementarity may not be required; in general, any Oligomer having sufficient complementarity to form a stable duplex (or triple helix complex as the case may be) with the target

nucleic acid is considered to be suitable. Since stable duplex formation depends on the sequence and length of the hybridizing Oligomer and the degree of complementarity between the antisense Oligomer and the target sequence, the system can tolerate less fidelity (complementarity) when longer Oligomers are used. This is also true with Oligomers which form triple helix complexes. However, Oligomers of about 8 to about 40 nucleosidyl units in length which have sufficient complementarity to form a duplex or triple helix structure having a melting temperature of greater than about 40°C under physiological conditions are particularly suitable for use according to the methods of the present invention.

The concentration of Oligomer used may vary, depending upon a number of factors, including the type of hyperproliferative condition to be treated, the tissue to be treated (i.e., whether administered locally or systemically), the type and the specificity of the particular antisense Oligomer selected. The studies described herein have observed significant inhibition in the test systems used at concentrations in the 50 μM range; however, under other conditions, higher or lower concentrations of Oligomer may be preferred.

For indications where Oligomers are to be administered transdermally, preferred are neutral Oligomers.

For indications where Oligomers are to be administered parenterally, such as by injection, either neutral Oligomers or Oligomers having an ionically charged backbone (i.e., having charged internucleosidyl linkages) may be used.

According to one preferred aspect, these Oligomers may comprise a conjugate between a polynucleoside or nucleoside/non-nucleoside polymer and a conjugation partner. Suitable conjugation partners include intercalating agents such as acridine, alkylating agents, binding substances for cell surface receptors, lipophilic agents, photo-crosslinking agents such as psoralen, other cross-

linking agents, pro-chelates, or nucleic acid modifying agents, including groups capable of cleaving a targeted portion of a nucleic acid such as hydrolytic or nucleolytic agents like o-phenanthroline copper or EDTA-iron, all of which may be incorporated in the Oligomers.

Conjugation partners may also be introduced into the Oligomer by the incorporation of modified nucleosides or nucleoside analogs through the use of enzymes or by chemical modification of the Oligomer, for example, by the use of non-nucleotide linker groups.

When used to prevent function or expression of a single or double stranded nucleic acid sequence, these Oligomers may be advantageously derivatized or modified to incorporate a nucleic acid modifying group which may be caused to react with said nucleic acid and irreversibly modify its structure, thereby rendering it non-functional.

Commonly assigned USSN 565,299, the disclosure of which is incorporated herein by reference, discloses psoralen-derivatized Oligomers.

As discussed above, a wide variety of nucleic acid modifying groups may be used as conjugation partners to derivatize these Oligomers. Nucleic acid modifying groups include groups which, after the derivatized Oligomer forms a complex which a single stranded or double stranded nucleic acid segment, may be caused to cross-link, alkylate, cleave, degrade, or otherwise inactivate or destroy the nucleic acid segment or a target sequence portion thereof, and thereby irreversibly inhibit the function and/or expression of that nucleic acid segment.

The location of the nucleic acid modifying groups in the Oligomer may be varied and may depend on the particular nucleic acid modifying group employed and the targeted nucleic acid segment. Accordingly, the nucleic acid modifying group may be positioned at the end of the Oligomer or intermediate between the ends. A plurality of nucleic acid modifying groups may be included.

In one preferred aspect, the nucleic acid modifying group is photoreactable (e.g., activated by a particular wavelength, or range of wavelengths of light), so as to cause reaction and, thus, cross-linking between the Oligomer and the nucleic acid target.

Exemplary of nucleic acid modifying groups which may cause cross-linking are the psoralens, such as an aminomethyltrimethyl psoralen group (AMT). The AMT is advantageously photoreactable, and thus must be activated by exposure to particular wavelength light before cross-linking is effectuated. Other cross-linking groups which may or may not be photoreactable may be used to derivatize these Oligomers.

Alternatively, the nucleic acid modifying groups may comprise an alkylating agent group which is covalently bonded to the nucleic acid segment to render it inactive. Suitable alkylating agent groups are known in the chemical arts and include groups derived from alkyl halides, haloacetamides and the like. Polynucleotide modifying groups which may be caused to cleave the polynucleotide segment include moieties which generate radicals, as well as moieties, which promote hydrolysis through nucleophilic attack. Transition metal chelating complexes, such as ethylenediaminetetraacetate (EDTA) or a neutral derivative thereof, can be used to generate radicals. Other groups which may be used to effect radical mediated cleavage include phenanthroline, porphyrin and the like. When EDTA is used, iron may be advantageously tethered to the Oligomer to help generate the cleaving radicals. Although iron-EDTA is a preferred polynucleotide cleaving group, other nitrogen containing materials, such as azo compounds or nitrenes or other transition metal chelating complexes may be used. Yet other cleavage agents include nucleophilic agents and hydrolytic agents that promote the addition of water at the phosphorus internucleotide linkages. Such agents include amines, substituted guanidinium groups, imidazole groups and the like.

1. Preferred Neutral Oligomer Formulations

Preferred neutral Oligomers include neutral alkyl- and aryl- phosphonate Oligomers and neutral Oligomers comprising morpholino or phosphoramidate linkages. Especially preferred are neutral methylphosphonate Oligomers. In view of their demonstrated ability to penetrate skin, including tape stripped skin, (which has had the stratum corneum removed and which has been reported as a model for mucous membrane), particularly preferred are neutral methylphosphonate Oligomers having only methylphosphonate internucleosidyl linkages.

Synthetic methods for preparing methylphosphonate Oligomers are described in Example 1 herein and also in Lee B.L., et al., Biochemistry 27:3197-3203 (1988), and Miller, P.S., et al., Biochemistry 25:5092-5097 (1986), the disclosures of which are incorporated herein by reference.

According to another aspect of the present invention, preferred are Oligomers which may be neutral until they enter cells and once inside are converted to charged species through chemical or biological processes. Such charged oligonucleotides may contain other moieties that stabilize the oligonucleotides to nuclease degradation. Substituents such as 2'-O-methylribose groups, various base modification, and analogs of the phosphorous backbone, such as phosphorothioates, can increase resistance to nucleases. Additionally, the presence of methylphosphonate or other neutral internucleoside linkages in the Oligomer give exonuclease resistance.

Preferred are neutral Oligomers having from about 6 to about 40 nucleosides, more preferably from about 12 to about 20 nucleosides. Although neutral Oligomers which comprise more than 20 nucleosides may be used, where complementarity to a longer sequence is desired, it may be advantageous to employ shorter neutral tandem Oligomers to maximize solubility and penetration through the skin or mucous membranes while competing for the development of a

secondary structure of the target nucleic acid, such as a mRNA. Alternatively, it may be advantageous to use more than one neutral Oligomer, each Oligomer complementary to a distinct target sequence which may be part of the same gene or a different gene.

Where the neutral Oligomers comprise alkyl- or aryl-phosphonate Oligomers, it may be advantageous to incorporate nucleoside monomeric units having modified ribosyl moieties. The use of nucleoside units having 2'-O-alkyl- and, in particular, 2'-O-methyl-ribosyl moieties in these neutral Oligomers may advantageously improve hybridization of the Oligomer to its complementary target sequence.

Suitable formulations comprise about 0.0001% to about 2% by weight of neutral Oligomer.

In one preferred aspect, there are provided neutral Oligomer formulations which comprise about 2% to about 100% of a short chain aliphatic alcohol. Suitable alcohols include ethanol, isopropyl alcohol, propylene glycol and glycerol. In certain studies, formulations of neutral Oligomers comprising ethanol have demonstrated advantageous transdermal flux.

In an especially preferred aspect, these neutral Oligomer formulations may additionally comprise a flux enhancer. Suitable flux enhancers include those known to those skilled in the art and include decylmethylsulfoxide, dimethylsulfoxide as well as cyclic ketones, lactones, anhydrides and esters such as those described in PCT Application No. PCT/US86/02583 (Publication Number WO87/03473). Some of these flux enhancers also increase retention of the Oligomer and, thus, act to increase the concentration of Oligomer within the skin itself.

Thus, for Oligomer formulations for direct (local) treatment, such as topical application to skin, it is preferred to use a flux enhancer which not only maximizes transdermal flux, but increases Oligomer retention in the skin. Certain cyclic ketone and lactone enhancers have been reported to increase local retention as well and,

thus, comprise a preferred class of enhancers for topical administration of Oligomer formulations.

In Oligomer formulations for systemic treatment, it is preferable to use a flux enhancer which maximizes flux with a minimal increase of local retention of Oligomer.

2. Preferred Target Genes and Target Sequences

According to a preferred aspect, the present invention is directed to methods of preventing or decreasing cellular proliferation using Oligomers which interfere with expression of cytokines which influence cellular proliferation, with conversion of such cytokines from a precursor to an active form, or with the expression of an intracellular receptor antagonist thereof. Suitable Oligomers include antisense Oligomers, Third Strand Oligomers and Triplex Oligomer Pairs.

According to one aspect of the present invention, there are provided methods of decreasing cellular hyperproliferation by preventing or interfering with expression of Interleukin-1β (IL-1β), Interleukin-1α (IL-1α) or intracellular splice variant of Interleukin-1 receptor antagonist ("intracellular Interleukin-1 receptor antagonist" or icIL-1ra) or a IL-1 converting enzyme, such as an IL-1β converting enzyme by administration of an Oligomer which is complementary to a target sequence on the DNA or a mRNA transcribed therefrom which codes for IL-1β, for IL-1α, for icIL-1ra, or for an IL-1β converting enzyme.

Thus, the present invention is directed to methods of decreasing hyperproliferation of keratinocytes or other epithelial cells by exposing the cells to a proliferation inhibiting amount of an Oligomer, either an antisense Oligomer, a Third Stand Oligomer or a Triplex Oligomer Pair. The antisense Oligomer is complementary to a sequence of RNA transcribed from a target gene. The Third Strand Oligomer has a base sequence selected so that it is capable of hydrogen bonding with a sequence of a double stranded nucleic acid and forming a triple helix complex

therewith. The first and second Oligomers of the Triplex Oligomer Pair have sequences selected such that they are complementary to and capable of hydrogen bonding with a targeted single-stranded nucleic acid sequence and together with the single stranded nucleic acid form a triple helix complex.

The target gene is selected from the group consisting of those genes encoding a cytokine which mediates cellular proliferation or a receptor antagonist or converting enzyme therefor.

Cytokines which comprise suitable targets include IL-1α and IL-1β. Since IL-1β is synthesized as a precursor of 31 K Daltons and for extracellular activity needs to be converted into an active form which is reported to be 17.5 K Daltons, a gene encoding an IL-1β converting enzyme is an alternate preferred target gene. Other alternate preferred target genes include those encoding other enzymes also involved in translational or post-translational modification of IL-1 family molecules that are critical for function of IL-1α, IL-1β or IL-1ra (for example, myristylation). Certain regions in the transcription product of the selected gene are preferred targets for the Oligomer.

Oligomers of appropriate length, preferably from about 8 to 40 nucleotides, more preferably from about 12 to about 20 nucleosides, are selected so as to be adjacent to or cover these sites when hybridized to the target, in part or in whole. Such sites include, in the pre-mRNA, splice acceptor, splice donor, and splice branch points, and polyA addition region. The preferred sites in mRNA include the initiation codon, or the 5′ end of the mRNA (cap site). The sequence of the Oligomers would be the reverse complement of the sequence of the targeted region.

As examples, in the case of IL-1β these sites would include the following, with reference to the nucleotide positions of the human IL-1β gene (GenBank accession number M15840):

Splice acceptor, donor junctions	nucleotide positions:	445/446; 908/909; 970/971; 1535/1536; 1587/1588; 3575/3576; 3777/3778; 4322/4323; 4487/4488; 5722/5723; 5853/5854; 6569/6570.
Poly A addition signal	nucleotide positions	7367-7372.
Initiation codon	nucleotide positions	924-926.
mRNA cap site	nucleotide positions	374.

As examples of preferred sites in the case of IL-1α, these sites and nucleotide positions with reference to the sequence numbers of human IL-1α gene (GenBank accession number X03833) include the following:

Splice acceptor, donor junctions	nucleotide positions	1488/1489; 2152/2153; 2207/2208; 3165/3166; 3214/3215; 4102/4103; 4325/4326; 6261/6262; 6432/6433; 7814/7815; 7939/7940; 10289/10290.
Poly A addition signal	nucleotide positions	11618-11623
Initiation codon	nucleotide positions	2161-2163
mRNA cap site	nucleotide positions	1438

The following site is an example of a preferred site of icIL-1ra, the site and nucleotide positions given are in reference to the nucleotide positions of intracellular

splice-variant of the human IL-1ra gene (GenBank accession number M55646):

Initiation codon nucleotide positions 123-125

Additional preferred target sites would comprise a mRNA cap site or splice junctions of the IL-1ra gene.

As examples of the preferred sites in IL-1β converting enzyme are the following, sites and nucleotide positions of the human IL-1β converting enzyme given in reference to that gene (GenBank accession number M87507):

Initiation codon	nucleotide positions	18-20
Poly A signal	nucleotide positions	1316-1321 or 1335-1340

Additional preferred target sites would include an mRNA cap or splice sites of the human IL-1β convertase.

Thus, according to a preferred aspect of the present invention, Oligomers of the appropriate length, preferably from about 8 to 40 nucleosides and more preferably from about 12 to about 20 nucleosides, are selected so as to have sequences which hybridize to sites immediately adjacent to these sites or hybridize with and cover these sites, in part or wholly, as defined by the nucleotide positions included above for IL-1β, IL-1α, icIL-1ra and IL-1β converting enzyme.

When antisense Oligomers are used the sequence of the Oligomers is the reverse complement of the sequence of the targeted region.

When Third Strand Oligomers are used, the Oligomers are selected to form sequence-specific hydrogen bonding interactions with the double stranded nucleic acid target.

When Triplex Oligomer Pairs are used, the first and second Oligomers are selected so as to form sequence specific hydrogen bonding interactions with a single stranded nucleic acid, and together form a triple helix structure.

3. Preferred Therapeutic Indications

Preferred therapeutic indications include conditions which can be classed as (a) cutaneous, benign hyper-proliferative; (b) cutaneous, malignant hyper-proliferative; (c) epithelial, benign hyper-proliferative; (d) epithelial, malignant hyper-proliferative; and (e) non-epithelial, hyper-proliferative.

Cutaneous conditions in which epidermal hyper-proliferation results in symptomatology include, but are not limited to, psoriasis, ichthyosis, pityriasis rubra pilaris ("PRP"), chronic dermatitis, psoriasiform dermatitis, atopic dermatitis, viral acanthoma (warts), other benign growths, lichen simplex chronicus, and mycosis fungoides/Sezary syndrome.

Malignant, cutaneous, hyper-proliferation of the epidermal keratinocytes include squamous cell carcinomas, basal cell carcinomas, actinic keratoses, keratoacanthomas and other epithelial neoplasms of the skin.

Epithelial, non-malignant hyper-proliferative conditions include oral mucosal, vaginal, cervical, esophageal, pulmonary and gastrointestinal hyperplasias and dysplasias, laryngeal papillomas and bladder cystitis.

Epithelial, malignant conditions of non-epidermal epithelial cells include squamous cell and other epithelial carcinomas of the head and neck, pulmonary tree, intestines, breast, bladder, cervix, uterus, and vagina.

Non-epithelial hyper-proliferations may also be responsive. These include rheumatoid arthritis, polycystic renal disease, restenosis and fibrotic conditions of various organs. Non-epithelial cancers may also be targets.

Also the methods of the present invention may be used in the treatment of conditions where preventing expression of IL-1 family of proteins results in secondary inhibition of release of other cytokines, whereby the condition is ameliorated. Such conditions include, but are not limited

to, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, uveitis or inflammation in psoriasis.

a. Role of IL-1α, IL-1β, and IL-1 Receptor Antagonist in Keratinocyte Growth

A major fundamental issue is whether psoriasis is an immunological disorder or the result of unregulated keratinocyte growth. IL-1 elicits activities in a wide variety of cell types that result in pathological effects that are seen in psoriasis. In addition, dysregulation of IL-1 has been reported in psoriatic tissue and may have consequences to the disease state. (Cooper, K.D. et al., J. Invest. Dermatol., 95:245-265 (1990)). Psoriatic tissue has been reported to have decreased levels of IL-1α (~1/10) and increased levels of IL-1β (~2x) over normal tissue (Cooper, K.D., et al., J. Immunol. 144:4593-4603 (1990)). Also, an intracellular form of the IL-1 receptor antagonist is expressed in normal epidermis and dominates functionally in psoriatic epidermis (Hammerberg, C., et al., "Interleukin-1 Receptor Antagonist in Normal and Psoriatic Epidermis," J. Clin. Invest., 1992 (in Press)), although the IL-1 receptor antagonist is responsible for the IL-1 inhibitory activity that dominates in psoriatic skin. (Kim, N-I, et al., "Psoriatic Skin Reveals the in vivo Presence of the Epidermal IL-1 Inhibitor," Arch. Dermatol. Res., 1991 (in Press)). In the epidermis, all members of the IL-1 family are present almost exclusively intracellularly and are not secreted, although they can be released. The cytokines are present as both precursor and product; however, IL-1β in normal or psoriatic tissue is processed into an inactive form as assayed by T-cell activation. These results indicate a potential autocrine role of IL-1 and/or IL-1ra in keratinocyte growth control either intracellularly or extracellularly. Furthermore, IL-1α, IL-1β and IL-1ra intracellular levels increase as keratinocytes enter a growth cycle; conversely, an abnormal keratinocyte population present in psoriasis skin

becomes blocked in S phase in association with a precipitous drop in IL-1α, IL-1β and IL-1ra levels (Hammerberg, C., Abstract, J.I.D., April, 1992).

To assess the intracellular role of IL-1α, IL-1β, and icIL-1ra in the growth of keratinocytes, antisense Oligomers provide one of the few methods to examine the functional importance of the gene products. Antisense inhibition of gene expression provides not only the specificity of discrimination between genes but also between highly related genes. Two different keratinocyte cultures have been used in these studies. The normal keratinocyte cultures are derived from normal human skin by separating the epidermis and culturing the keratinocytes (Baadsgard, O., et al., J. Invest. Dermatol. 95:275-282 (1990)). Another culture is a keratinocyte cell line that retains the properties of keratinocytes and can undergo terminal differentiation to form stratum corneum when transplanted into nude mice (Boukamp, P., et al., J. Cell. Biol. 106:761-771 (1988)).

b. Inhibition of Keratinocyte Proliferation

Inhibition of gene expression of IL-1β, IL-1α and IL-1ra was studied using Oligomers complementary to portions of the IL-1β, IL-1α or icIL-1ra mRNA. Two different keratinocyte cell lines were used in these studies. The normal keratinocyte cultures were derived from normal human skin by separating the epidermis and culturing the keratinocyte cells. (See Baadsgard et al., supra.) A second cell culture was a keratinocyte cell line that retained the properties of keratinocytes and which can undergo terminal differentiation to form stratum corneum when transplanted into nude mice (HaCaT cell line). (See Barkamp et al, supra.)

Antisense Oligomers used in these studies were directed to the initiation codon of the individual gene target mRNA's or a splice junction and are shown in

Table I along with additional sequences that have been used to assess sequence specificity.

Table I

Methylphosphonate Oligomers

Oligomer#	Target	Sequence	Site
1 [1252]	human IL-1β	TTC-TGC-CAT-GGC-TGC	initiation codon
2 [1421]	human IL-1β	CAC-CTG-TGT-AAA-AAG	splice site
3 [1251]	human IL-1α	GCC-ATC-TTG-ACT-TCT	initiation codon
4 [1487]	human IL-1 receptor antagonist (intracellular splice-variant)	GCC-ATG-GGG-AGG-GCC	initiation codon
C1 [1480]	mismatch to Oligomer #1 [1251]	TTG-TGC-CAT-GCC-TGC	2 nucleotide mismatch
C3 [1588]	mismatch to Oligomer #3 [1588]	GCG-ATC-TTC-ACT-TCT	2 nucleotide mismatch
C4 [1607]	mismatch to Oligomer #4 [1487]	GCG-ATC-GGG-ACG-GGC	4 nucleotide mismatch

From a review of the results reported in Examples 2 and 3 herein, expression of the IL-1α or IL-1β cytokines or IL-1ra in keratinocytes appears to be an essential function for their proliferation. The constitutive expression of these cytokines by keratinocytes and their intracellular localization suggests that the effects of the antisense Oligomers are on new continual synthesis. The capacity to limit or reduce the proliferation of keratinocytes in culture by the use of sequence specific antisense Oligomers indicates that these Oligomers will be

useful to limit proliferation of keratinocytes in diseases such as psoriasis. In addition to IL-1α and IL-1β, keratinocytes also produce a number of other cytokines that may lead to inflammatory cell infiltration of the dermis. Keratinocytes in psoriasis release a balance of cytokines that boost T-cell activation. (Chang, E.Y., "T-Cell Activation is Potentiated by Cytokines Released by Lesional Psoriatic, But Not Normal, Epidermis," Arch. Dermatol., Submitted, 1992). It might be expected that inhibiting keratinocyte proliferation may also have an effect upon the immune component of the disease, not only because the IL-1 group is one of several synergizing cytokines responsible for boosting T-cell activation in psoriasis, but because changing the intracellular cytokine milieu by use of an antisense or Third Strand Oligomer to IL-1β or IL-1α one may also block release of other cytokines.

To assist in understanding the present invention, the following examples are included which describe the results of a series of experiments. The following examples relating to this invention should not, of course, be construed in specifically limiting the invention and such variations of the invention, now known or later developed, which would within the purview of one skilled in the art are considered to fall within the scope of the present invention as hereinafter claimed.

Examples

Example 1

Preparation of Methylphosphonate Oligomers

Neutral methylphosphonate Oligomers are synthesized using methylphosphonamidite monomer, according to the chemical methods described by P.S. Miller et al. (Nucleic Acids Res. 11:6225-6242 (1983)), A Jager and J. Engels (Tetrahedron Letters 25:1437-1440 (1984)) and M.A. Dorman et al. (Tetradhedron Letters 40:95-102 (1984)).

Solid phase synthesis is performed on a Milligen Model 8800 DNA synthesizer. The programs used with the

synthesizer are named MTHL_06 (main) and CPLAW11 (coupling) and are obtained from the manufacturer.

The reagent mixtures used are as follows:

1. Activator: 0.45 M tetrazole in acetonitrile.
2. Cap A: 40% acetic anhydride in acetonitrile.
3. Cap B: 0.625% dimethylaminopyridine in pyridine.
4. Deblock: 2.5% dichloroacetic acid in dichloromethane.
5. Oxidizer: 0.1 M I_2 in tetrahydrofuran/2,6-lutidine/water (74.82/25/0.18; v/v/v).
6. Wash A: acetonitrile containing less than 30 ppm water.
7. Wash B: acetonitrile containing less than 30 ppm water.
8. Monomers: all monomers and diluted to 0.08 M in acetonitrile.
9. Support: The Oligomer is synthesized using a support acrylate beads derivatized with the appropriate nucleoside.

The crude, protected methylphosphonate Oligomers are removed from the solid support by mixing with acetonitrile/ethanol/concentrated ammonium hydroxide (45/45/10; v/v/v) for thirty minutes at room temperature. Next, the protecting groups are removed from the bases by addition of an equal volume of ethylenediamine (high quality) for 6 hours at room temperature. The resulting solution is diluted 10-fold with water and then neutralized with glacial acetic acid.

The solution containing Oligomers is passed over a Sep-Pak™ C18 cartridge (Millipore/Waters Bedford, MA) prepared according to the manufacturer's specification. The column is washed with water and the Oligomers are eluted with 50% acetonitrile in water.

The methylphosphonate Oligomers are further purified by, for example, reverse-phase HPLC chromatography as follows: A Beckman System Gold HPLC is used with a What-

man RAC II ODS-3 column (5 μ, 9 mm i.d. x 100 mm long). Buffer A= 50 mM triethylammonium acetate (pH 7); Buffer B=50% acetonitrile in 50 mM triethylammonium acetate (pH 7). The sample, dissolved in 10% acetonitrile/water, is loaded onto the column using an external pump. Next, the column is attached to the Beckman HPLC system and a gradient of 0 to 20% Buffer B over 5 minutes, followed by a gradient of 20 to 60% Buffer B over 40 minutes, is run at a flow rate of 3.0 ml/minute. Fractions are collected and those containing full length methylphosphonate Oligomer are pooled, evaporated under vacuum, and resuspended in 50% acetonitrile/water.

Example 2

Inhibition of Proliferation of Normal Keratinocytes Using Antisense Oligomers

Normal human keratinocytes were obtained by the procedure described by Baadsgaard et al. (J. Invest. Dermatol. 95:275-282 (1990)) and grown in keratinocyte growth medium (Clonetics, San Diego, CA).

Oligomer stocks were in 50% acetonitrile/H_2O and were diluted at least 100 fold to achieve a concentration of 100μM, in the culture medium, unless noted otherwise. The maximum amount of acetonitrile in the media was 0.5%.

Cells were plated at 3 X 10^3 cells in a single well of a 96-well cell culture plate. The cells were maintained at 37°C in a CO_2 incubator. After 4 days of growth, unless otherwise indicated, ^{3}H-dT (1μCi) was added and the label was incorporated for 6 hours. The acid-precipitable label was collected and the counts determined using a scintillation counter. For each treatment or control, six wells (replicates) were setup and run. Of the six replicates, the ones having highest and lowest number of counts were eliminated and the average value and error were calculated using the remaining four values.

Treatment of freshly obtained normal keratinocytes cultured ex vivo with methylphosphonate (MP) antisense Oligomer to IL-1α or IL-1β was carried out for different lengths of time to evaluate the effect on growth when the cytokines are targeted. The results indicate targeting either IL-1α or IL-1β inhibits the proliferation (as measured by ^{3}H-thymidine incorporation into DNA) of normal keratinocytes in culture (Table II) and the extent of inhibition is dependent upon length of treatment and demonstrates a decrease in the rate of proliferation of the cells with antisense treatment. Oligomers targeted at either the IL-1β initiation codon region or a splice site both had similar inhibitory activity.

Table II

Inhibition of Normal Keratinocyte Growth by MP-Oligomer With Time of Treatment

MP Oligomer	Time (days)	^{3}H-dT incorp.	Inhibition of growth[1/]
Control (no Oligomer)	2	4,900 ± 487	---
	4	2,679 ± 180	---
	7	2,157 ± 1,476	---
1 [IL-1β (1252-2)]	2	3,723 ± 340	24%
	4	1,583 ± 497	41%
	7	662 ± 231	69%
2 [IL-1β (1421-1)]	2	2432 ± 171	51%
	4	1347 ± 191	50%
	7	861 ± 218	60%
3 [IL-1α (1251-2)]	2	3,089 ± 93	37%

1/ Relative to same day control.

Table II (cont'd)

MP Oligomer	Time (days)	^{3}H-dT incorp.	Inhibition of growth
	4	1,141 ± 111	57%
	7	652 ± 88	70%

To examine the sequence specificity of inhibition of proliferation, a methyl phosphonate ("MP") Oligomer was synthesized with the same base composition as an IL-1β Oligomer (1252) but 2 nucleotides were exchanged in their position in the molecule, thus, the Oligomer would be expected to have low or no affinity for the targeted gene. The Oligomers were incubated with normal keratinocyte cultures for 4 days at 100 μM. Inhibition of normal keratinocyte proliferation by the exact match Oligomer to IL-1β [Oligomer 1 (1252)] was 54% whereas the mismatch Oligomer [Oligomer C1 (1480)] did not inhibit proliferation and showed increased incorporation that was within the standard error of the assay (Table III). The inhibition of normal keratinocyte proliferation that was seen for Oligomer 1 was sequence specific in that exchange of two nucleotides in the sequence of Oligomer C1 abolished any inhibitory effects of added Oligomer.

Table III

Specificity of IL1 β Inhibition of Normal Keratinocytes by MP-Oligomer (100 μM)

MP Oligomer	Time	^{3}H-dT incorp.	Inhibition of growth
Control (no Oligomer)	4 days	8,028 ± 1,169	---
1 [IL-1β (1252-2)]	4 days	3,713 ± 930	54%
C1 [Mismatch to IL-1β (1480-1)]	4 days	8,737 ± 3,113	(+10%)

Example 3

Inhibition of Proliferation of a Keratinocyte Cell Line Using Antisense Oligomers

An established human keratinocyte cell line HaCaT has also been used to provide a continuous source of cells. The cell line maintains some of the properties of keratinocytes most notably the capacity to terminally differentiate when transplanted onto immuno-compromised mice (Boukamp, P., et al., J. Cell. Biol. 106:761-771 (1988)).

The HaCaT cell line was maintained in DMEM with 10% fetal calf serum.

Oligomer stocks were kept and diluted as described in Example 2. The cells were plated, incubated, labelled and precipitable label determined as described in Example 2.

Concentration dependence of IL-1β Oligomer (Oligomer 1) inhibition of HaCaT cells after 4 days at the indicated concentration of Oligomer is shown in Table IV along with Oligomer C1, the mismatch Oligomer. A dose dependent inhibition of HaCaT cells by antisense IL-1β Oligomer (Oligomer 1) was observed, whereas no decrease in proliferation was seen with Oligomer C1, which had a 2 nucleotide mismatch. The keratinocyte cell line showed similar inhibition to that observed with normal human keratinocytes by the antisense IL-1β (Oligomer 1 (1252)).

Table IV

Inhibition of HaCaT Keratinocyte Cell Line by MP-Oligomer Against IL-1β

MP Oligomer	Conc μM	^{3}H-dT incorp.	Inhibition of growth
Control + vehicle (no Oligomer)		195,497 ± 22,445	--
Control (no Oligomer)		187,572 ± 8,638	

Table IV (cont'd)

MP Oligomer	Conc μM	^{3}H-dT incorp.	Inhibition of growth
1 [IL-1β (1252-3)]	100	14,104 ± 2,338	93%
	50	75,116 ± 9,254	61%
	25	157,513 ± 10,142	19%
	12.5	160,206 ± 14,198	18%
	6.2	191,573 ± 6,588	2%
C1 [Mismatch to IL-1β (1480-1)]	100	153,749 ± 12,035	21%
	50	177,304 ± 14,682	9%
	25	189,813 ± 4,653	3%
	12.5	188,146 ± 15,618	4%
	6.2	191,573 ± 6,588	2%

Example 4

Inhibition of Proliferation of a Cell Line Using Antisense Oligomers to IL-1α

Oligomers and HaCaT cells were treated as described in Example 3.

Concentration dependence of IL-1α Oligomer [Oligomer 3 (1251-3)] treatment of HaCaT cells after 4 days at concentrations of Oligomer from 0 to 100 μM along with treatment with an antisense IL-1α Oligomer [Oligomer C3 (1588-1)] having 2 nucleotide mismatch was carried out.

The role of IL-1α in inhibition of growth of the HaCaT cell line observed in this experiment was less clear than that described in Example 2 using normal keratinocytes. Addition of the antisense IL-1α Oligomer (Oligomer 3) did not result in any significant decrease in the growth of the HaCaT cells and was similar in effect to the mismatch control Oligomer C3. This finding reflects a difference between the HaCaT cell line and cultured human keratinocytes.

Example 5

Inhibition of Proliferation of Keratinocytes by Antisense Oligomers to Intracellular IL-1RA

Oligomers and cells were treated as described in Examples 2 and 3.

Intracellular IL-1 receptor antagonist (icIL-1ra) has been targeted by antisense MP Oligomers to examine its role in keratinocyte proliferation. (See, e.g., Eisenberg, S.P., et al., Nature 343:341-346 (1990); Hannum, C.H., et al., Nature 343:336-340 (1990); Kupper, T.S., J. Clin. Invest. 86:1783-1789 (1990)). The intracellular IL-1ra has a initiation codon region that is unique to it and not to the soluble form of IL-1ra. This region provides a unique target for discriminating between the intracellular and soluble forms of the gene products. Incubation of the antisense MP Oligomer to the icIL-1ra with normal human keratinocytes resulted in a strong inhibition of growth (See Table V) of >90%. The icIL-1ra may have a similar function in the cell to IL-1β as there is strong homology between the two proteins in their amino acid sequences (March, C.J., et al., Nature (Lond.) 315:641 (1985)), and icIL-1ra may not have a role as a receptor antagonist within the cell.

Table V

Inhibition of Normal Keratinocyte Growth With MP Oligomer Against IL-1 Receptor Antagonist

MP Oligomer	Time (days)	^{3}H-dT incorp	Inhibition of growth[1]
Control (no Oligomer)	2	6,630 ± 1,218	---
	4	13,981 ± 2,722	---
4 [IL-1ra intracellular splice variant (1487-2)]	2	479 ± 78	92%
	4	293 ± 18	97%

[1] Relative to same day control

Examination of the role of icIL-1ra in the keratinocyte culture was carried out using different concentrations of Oligomer 4 (1487) with HaCaT cells (See Table VI). At the highest concentration, after 4 days of incubation, antisense Oligomer to icIL-1ra (Oligomer 4) inhibited proliferation by 60%. However, decreasing concentrations of Oligomer 4 did not indicate a gradual decrease, but an abrupt change in the effectiveness of the agent occurred between 12.5 and 6.25 μM Oligomer. The inhibition of HaCaT cells at the highest concentration of Oligomer 4 was similar to that seen with the normal keratinocyte cultures (Table V). The mismatch Oligomer, Oligomer C4 (1607) which had 4 nucleotides switched from Oligomer 4 (1487) did not inhibit keratinocyte proliferation. The results of Tables V and VI suggest that the icIL-1ra may also play an functional role in the regulation of keratinocyte proliferation.

Table VI

Inhibition of HaCaT Cells With MP-Oligomer Against the IL1 Receptor Antagonist

MP Oligomer	Conc μM	^{3}H-dT incorp.	Inhibition of growth
Control + vehicle (no Oligomer)		130,188 ± 18,795	---
Control (no Oligomer)		109,264 ± 163	
4 [IL-1ra intracellular splice variant (1487-3)]	100	49,518 ± 7,840	62%
	50	120,073 ± 11,537	8%
	25	109,852 ± 26,472	16%
	12.5	97,371 ± 35,155	25%
	6.2	166,954 ± 9,994	(+28%)
C4 [Mismatch to IL-1ra intracellular splice variant (1607-1)]	100	153,711 ± 37,747	(+18%)
	50	158,493 ± 6,700	(+20%)
	25	170,470 ± 17,767	(+31%)
	12.5	139,990 ± 30,983	(+8%)
	6.2	186,836 ± 26,101	(+43%)

Claims

1. A method of treating a pathologic condition characterized by hyperproliferation of skin or epithelial cells which comprises:

exposing skin or epithelial cells to a hyperproliferation inhibiting amount of an Oligomer selected from (a) an antisense Oligomer having a sequence complementary to a sequence of RNA transcribed from a target gene present in the cells; (b) a Third Strand Oligomer having a sequence complementary to a selected double stranded nucleic acid sequence of a target gene present in the cells, and (c) a Triplex Oligomer Pair which is complementary to a single stranded nucleic acid sequence of a target gene or its transcription product wherein said target gene is selected from the group consisting of those genes encoding a cytokine which mediates cellular proliferation, a modulating factor for the cytokine, a converting enzyme which converts a precursor form of the cytokine to the active cytokine, or an enzyme involved in translational or post-translational modification of said cytokine critical for its function, whereby hyperproliferation of the cells is decreased.

2. A method according to claim 1 wherein said target gene modulates cellular proliferation through an intracellular mechanism.

3. A method according to claim 2 wherein said target gene encodes IL-1α, IL-1β or icIL-1ra, or an IL-1β converting enzyme.

4. A method according to claim 2 wherein said pathologic condition comprises benign hyperproliferation of keratinocytes.

5. A method according to claim 2 wherein said pathologic condition comprises malignant hyperproliferation of keratinocytes.

6. A method according to claim 2 wherein said pathologic condition comprises benign hyperproliferation of non-epidermal epithelial cells.

7. A method according to claim 2 wherein said pathologic condition comprises malignant hyperproliferation of non-epidermal epithelial cells.

8. A method according to claim 2 wherein said pathologic condition is psoriasis.

9. A method according to claim 2 wherein said pathologic condition comprises a chronic inflammatory disease which is perpetuated by cytokine release regulated by IL-1α, IL-1β or icIL-1ra production.

10. A method according to claim 9 wherein said chronic inflammatory disease is selected from rheumatoid arthritis, inflammatory bowel disease, psoriasis, and inflammatory ocular disease.

11. A method according to claim 1 wherein said Oligomer is a neutral Oligomer.

12. A method according to claim 1 wherein said Oligomer is an antisense Oligomer.

13. A method according to claim 1 wherein said Oligomer is a Third Strand Oligomer or a Triplex Oligomer Pair.

14. A method of preventing or decreasing hyperproliferation of skin or epithelial cells which comprises:

exposing skin or epithelial cells to a hyperproliferation-inhibiting amount of an Oligomer selected from (a) an antisense Oligomer having a sequence complementary to a sequence of RNA transcribed from a target gene present in the cells; (b) a Third Strand Oligomer having a sequence complementary to a selected double stranded nucleic acid sequence of a target gene present in the cells, and (c) a Triplex Oligomer Pair which is complementary to a single stranded nucleic acid sequence of a target gene or its transcription product wherein said target gene is selected from the group consisting of those genes encoding a cytokine which mediates cellular proliferation, a modulating factor for the cytokine, a converting enzyme which converts a precursor form of the cytokine to the active cytokine, or an enzyme involved in translational or post-translational modification of said cytokine critical for its function.

15. A method according to claim 14 wherein said cytokine or receptor antagonist mediates cellular proliferation through an intracellular mechanism.

16. A method according to claim 14 wherein said Oligomer is a neutral Oligomer.

17. A method according to claim 14 wherein Oligomer is an antisense Oligomer.

18. A method according to claim 14 wherein said Oligomer is a Third Strand Oligomer.

19. A method of preventing or decreasing hyperproliferation of cells which comprises contacting said cells with a proliferation inhibiting amount of an Interleukin-1 inhibitory compound which decreases Interleukin-1 or intracellular Interleukin-1 receptor antagonist activity.

20. A method according to claims 19 wherein said cells are epithelial cells.

21. A method according to claim 20 wherein said Interleukin-1 inhibitory compound is selected from compounds which prevent or decrease Interleukin-1 expression or which inhibit or decrease conversion of a precursor form of Interleukin-1 into an active form.

22. A method according to claim 21 wherein said Interleukin-1 inhibitory compound inhibits or decreases synthesis of an Interleukin-1 converting enzyme or inhibits activity of the Interleukin-1 converting enzyme.

23. A method according to claim 19 wherein the Interleukin-1 inhibitory compound is an Oligomer which prevents or decreases expression of an Interleukin-1, an Interleukin-1 modulating factor, or an Interleukin-1 converting enzyme.

24. A method according to claim 19 wherein said Interleukin-1 inhibitory compound is selected from compounds which decrease intracellular Interleukin-1 receptor antagonist activity.

25. A pharmaceutical composition which comprises a hyperproliferation inhibiting amount of an Oligomer selected from (a) an antisense Oligomer having a sequence complementary to a sequence of RNA transcribed from a target gene present in the cells; (b) a Third Strand

Oligomer having a sequence complementary to a selected double stranded nucleic acid sequence of a target gene present in the cells, and (c) a Triplex Oligomer Pair which is complementary to a single stranded nucleic acid sequence of a target gene or its transcription product wherein said target gene is selected from the group consisting of those genes encoding a cytokine which mediates cellular proliferation, a modulating factor for the cytokine, a converting enzyme which converts a precursor form of the cytokine to the active cytokine, or an enzyme involved in translational or post-translational modification of said cytokine critical for its function and a pharmaceutically acceptable carrier.

26. A composition according to claim 25 wherein the target gene encodes IL-1α, IL-1β, icIL-1ra or an IL-1β converting enzyme.

27. A composition according to claim 25 wherein said Oligomer is a neutral Oligomer.

28. A composition according to claim 27 which further comprises a flux enhancer.

29. A composition according to claim 27 wherein the target gene encodes IL-1α, IL-1β, icIL-1ra or an IL-1β converting enzyme.

30. A composition according to claim 29 which further comprises a flux enhancer.

31. A pharmaceutical composition which comprises a hyperproliferation inhibiting amount of an Interleukin-1 inhibitory compound which decreases Interleukin-1 or intracellular Interleukin-1 receptor antagonist activity and a pharmaceutically acceptable carrier.

32. A composition according to claim 31 wherein the Interleukin-1 inhibitory compound is selected form compounds which prevent or decrease Interleukin-1 expression or which inhibit or decrease conversion of a precursor form of Interleukin-1 into an active form.

33. A composition according to claim 31 wherein said Interleukin-1 inhibitory compound inhibits or decreases synthesis of an Interleukin-1 converting enzyme or inhibits activity of the Interleukin-1 converting enzyme.

34. A composition according to claim 31 wherein the Interleukin-1 inhibitory compound is selected from compounds which decrease intracellular IL-1ra activity.

35. A composition according to claim 31 wherein the Interleukin-1 inhibitory compound is an Oligomer which prevents or decreases expression of an Interleukin-1, an Interleukin-1 modulating factor, or an Interleukin-1 converting enzyme.

36. A composition according to claim 35 wherein said Oligomer is a neutral Oligomer.

37. A composition according to claim 36 which further comprises a flux enhancer.

38. A composition according to claim 31 wherein said Interleukin-1 inhibitory compound is an Oligomer.

39. A composition according to claim 38 wherein said Oligomer is a neutral Oligomer.

40. A composition according to claim 39 which further comprises a flux enhancer.

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US93/04917

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/70; C07H 15/12, 17/00
US CL :514/44; 536/24.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 241, issued 22 July 1988, M. Cooney et al, "Site-Specific Oligonucleotide Binding Represses Transcription of the Human c-myc Gene in Vitro", pages 456-459, see abstract.	1-40
Y,P	US, A, 5,135,917 (Burch) 04 August 1992, col. 1, lines 22-23 and 65-68 and col. 8, lines 46-48.	1-40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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